

Two-Photon Fluorescence Microscopy for Biomedical Research

Overview

- Two-photon microscopy is a laser scanning imaging modality that allows high-resolution, three-dimensional imaging of relatively thick (400 μm) organic and inorganic fluorescent samples. This includes samples that are intrinsically fluorescent as well as those that have been stained with fluorescent dyes or prepared with fluorescent nanoparticles.
- Two-photon microscopy can be used to image cellular structure as well as fluorescent nanoparticle activity and physiological state. It can also be used to study bulk tissue.
- Two-photon can also be used to study diffusion and transport in microfluidic devices.
- Conventional (wide field) fluorescence microscopy is limited to thin samples at high-resolution due to short depth of focus (results in out-of-focus blur for thicker samples).
- Confocal microscopy allows high-resolution, three-dimensional imaging, but suffers from background fluorescence, as well as problems associated with the effects of ionizing radiation (harmful to living cells).
- Two-photon microscopy addresses these issues.

Background

- Unlike one-photon fluorescence microscopy, two-photon microscopy utilizes the simultaneous non-linear absorption of two near-infrared photons.
- The efficiency of two photon absorption is much lower than one-photon absorption.
 - Need ultra-fast pulsed laser
 - The nonlinear nature of the interaction implies that two-photon absorption will occur most strongly in the focal region.
 - Inherent optical sectioning

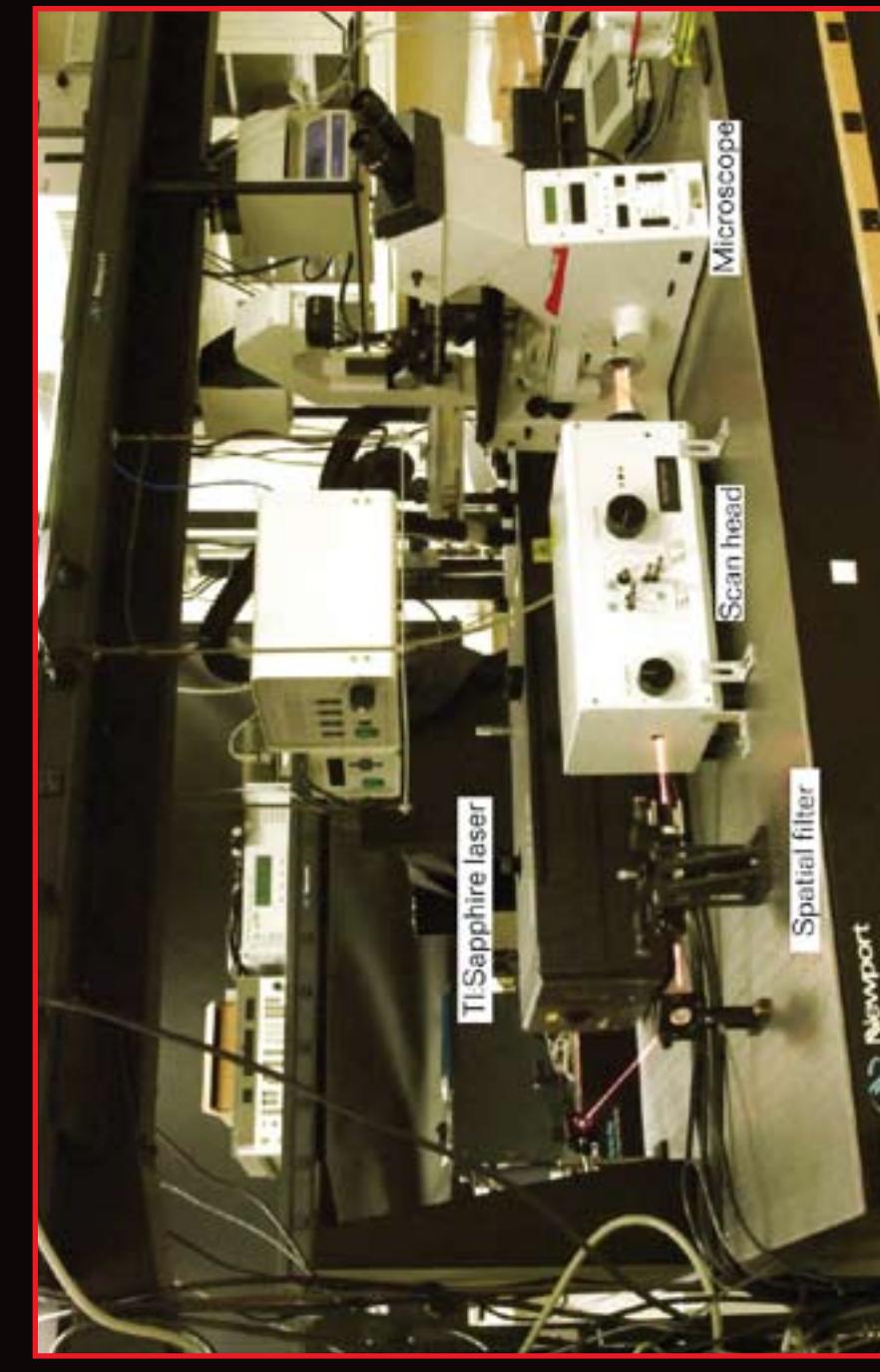
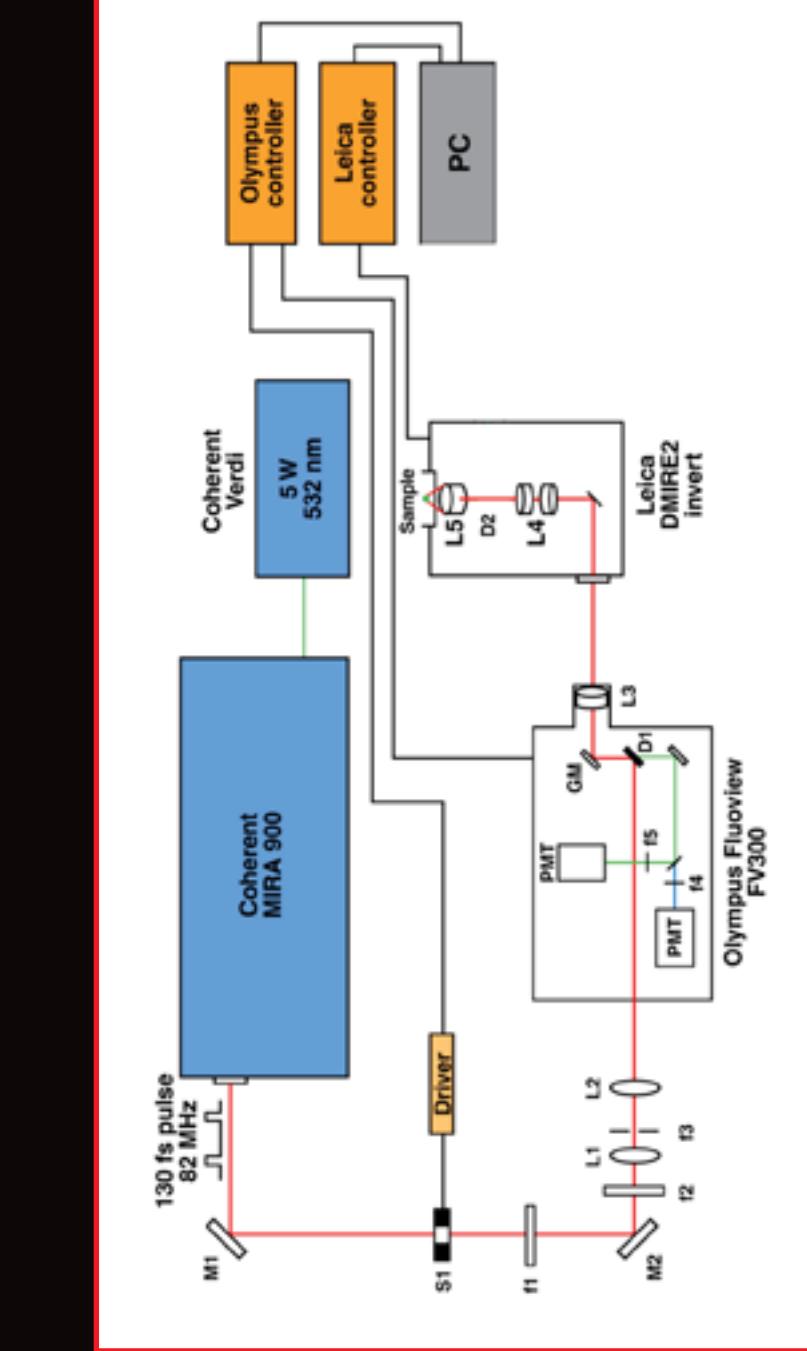
Principles of Operation

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Advantages Over Confocal

- Increased penetration depth due to the longer excitation wavelength
- Reduced harm to living cells
- Reduced background noise since no excitation outside focal volume

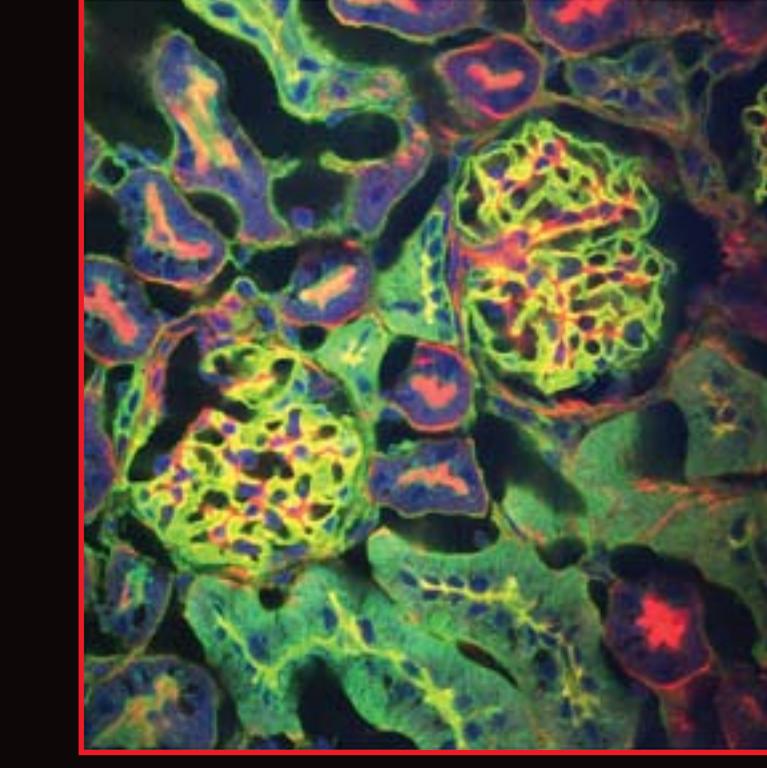
Modes of Operation



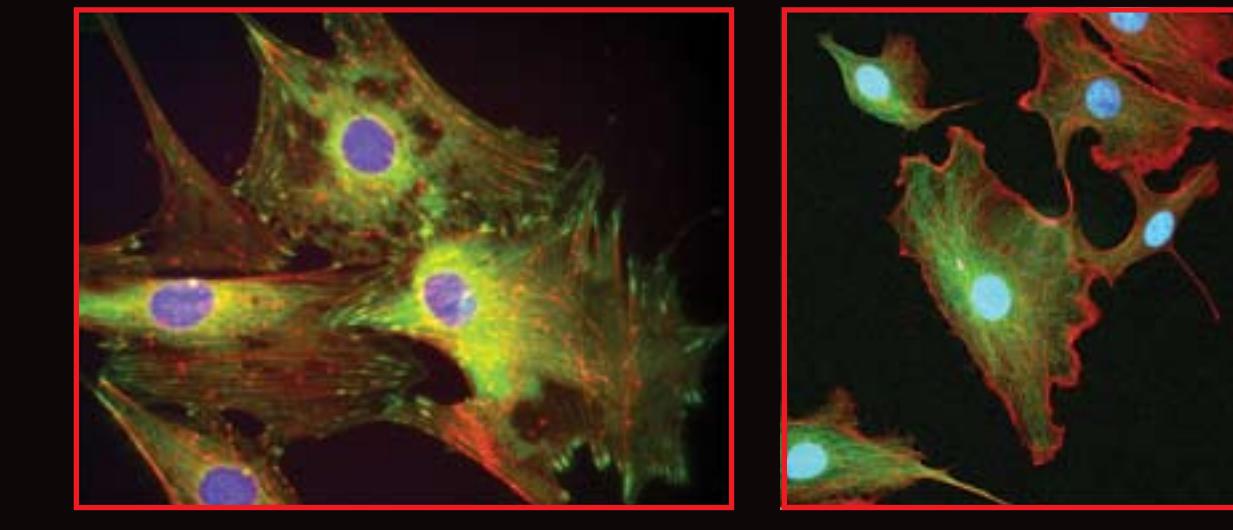
GRC Two-Photon System

Applications

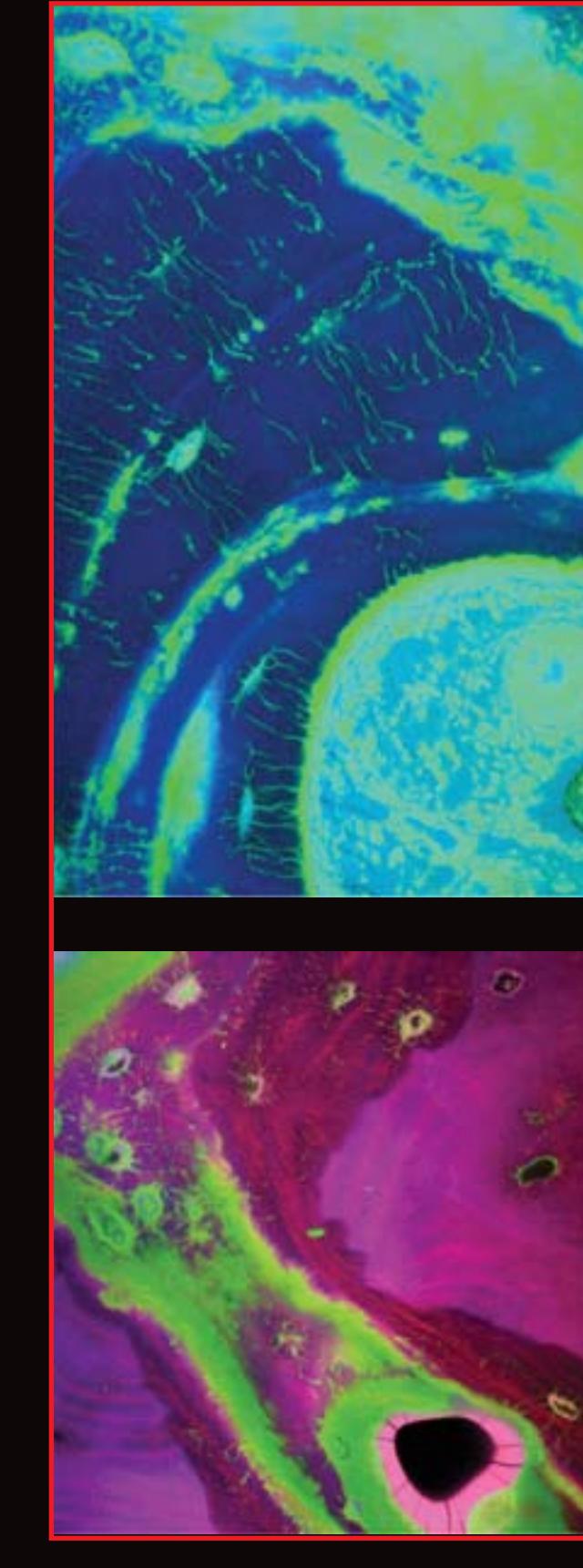
- Imaging
 - Fluorescence correlation spectroscopy (FCS)
 - Intracellular protein dynamics
 - Diffusion of fluorophores through nano-porous glass substrates (possible biosensor platform)
 - Fluorescence lifetime imaging microscopy (FLIM)
 - Local cellular (chemical) environment
 - Fluorescence resonance energy transfer (FRET)
 - Calcium signaling
 - Molecular interaction and structural change
 - Microscope has transverse resolution of 320 nm and an axial resolution of 740 nm (for 780 nm excitation)
 - Microscope is fitted with an incubated stage for live cell imaging
- Bone biology (bone loss)
- Cell sorting (wound healing)
- Intracellular dynamics (calcium signaling, protein interactions, diffusion/transport)
- Biosensor development
- Nanoparticle toxicology (in vitro studies)



Mouse osteoblast cells



Mouse kidney tissue section


 Human femur bone sections
Bovine pulmonary artery endothelial cells
